Northeast Gulf Science Vol. 3, No. 2, December 1979
Published April 25, 1980

A SIMPLE METHOD FOR STAINING THE CENTRA OF TELEOST VERTEBRAE

Use of vertebrae to study age and growth of teleost fishes has proved to be a valuable method by several authors (Menon, 1950; Mather and Schuck, 1960; Chadwick, 1976; Berry, Lee and Bertolino, 1977). In the past, processing vertebrae for age analysis has run the gamut from simple cleaning and drying to more complex methods such as with alizarin red S (Galtsoff, 1952). The goal of staining processes is to accentuate the growth "rings" on the centrum surface so that the rings are more visible and easier to count and measure in aging studies.

To study vertebrae of some marine fishes, I have developed a staining method which may be useful to others sampling fish vertebrae for age and growth analysis.

1. Separate vertebrae (fresh, frozen, or preserved in 5-10% Formalin) from each other and remove the gelatinous material from the centrum. Soaking vertebrae in sodium hypochloride solution (concentration and time dependent on species) will help remove the gelatinous material in difficult to clean vertebrae.

2. Cover vertebrae with a solution of 0.01% crystal violet (histological grade) in distilled water, stain for 0.2 to 4 hr depending on size of the vertebrae and the desired stain intensity.

3. Rinse stained vertebrae in water and air dry. Unpreserved large vertebrae may require preservation in 5% Formalin for 24 hr after staining to prevent decomposition.

4. Overstained vertebrae can be destained with 50% aqueous isopropanol. Centra surfaces after staining and drying with this technique have a satiny purple sheen with very visible rings

(Figure 1). With this method, I have successfully treated vertebrae of bluefin tuna *(Thunnus thynnus)*, king mackerel *(Scomberomorus cavalla)*, Spanish mackerel *(S. maculatus)*, blue fish *(Pomatomus saltatrix)*, and spotted seatrout *(Cynoscion nebulosus)*.

Seven stains were tested to develop this method (Table 1). Alizarin red S (0.32%) and crystal violet (0.01%) were most useful for staining centra.

Table 1. Results of various stains for staining centra of vertebrae.

Stain	Solvent	Remarks
Methylene Blue	Water	Tissue stained, but not centra.
Rose Bengal	Water	Tissue stained, but not centra.
Trypan Blue	Water	Tissue stained, but not centra.
Ponceau S	Water	Tissue stained, but not centra.
Amido Black	Water	Tissue and centra stained but staining intensity difficult to control.
Crystal Violet	Water	Tissue and centra stained; staining intensity controllable via concentration and time.
Alizarin Red S[1]		Centra stained, centra surface of some species damaged by process.

1/Method of Berry, Lee, and Beriolino, 1977.

For example, detailed comparison was made between the alizarin red S and crystal violet methods using a Gulf of Mexico bluefish (♀, 752 mm fork length, 6 years old based on scale aging).

Results were:

Alizarin red S (0.32%)

1 hr - Centrum surface light pink, distal edge not stained

3 hr - Centrum surface dark red, distal edge of each growth ring slightly lighter than rest of growth ring

5 hr - Centrum surface stained a uniform reddish purple

Crystal violet

1 hr - With a 0.01% solution, centrum surface completely stained a uniform purple

1 hr - With a 0.005% solution, centrum surface stained a light purple, distal edge of each growth ring had a slightly deeper hue than rest of growth ring

3 hr - With a 0.005% solution, centrum surface completely stained a uniform dark purple.

Microscopic examination at 500X of the centra surfaces stained with both alizarin red S and crystal violet, revealed three structural features in the bluefish vertebrae: ridges (growth rings; grooves (area between the growth rings); and fine concentric lines (present within ridges and grooves). Six ridges were

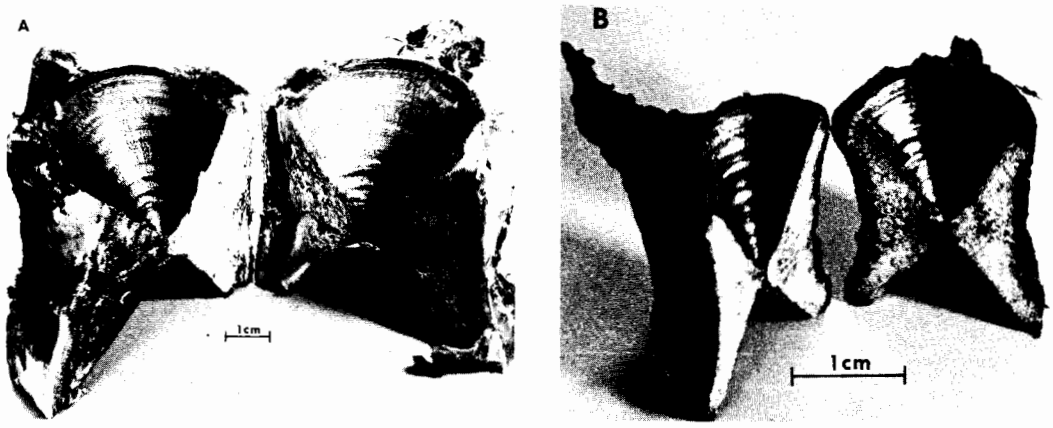

Figure 1. Vertebrae of (A) bluefin tuna and (B) bluefish stained with crystal violet.

found on all centra surfaces. Surfaces of the bluefish centra and their absorption of stain appeared similar to those of bluefin tuna (Atlantic) described by Berry *et al.* (1977).

The crystal violet method was less complicated and more rapid for staining vertebrae than the other stains.

ACKNOWLEDGMENTS

I thank Howard Horton (Oregon State University, Department of Fisheries and Wildlife, Corvallis, Oregon), Charles Manooch (National Marine Fisheries Service, Beaufort, North Carolina), and Michael Chadwick, (Environment Canada, Fisheries and Marine, St. John's Newfoundland, Canada) for their helpful reviews of this note. Special thanks are extended to Frederick Berry (National Marine Fisheries Service, Miami, Florida) for samples and information.

LITERATURE CITED

Berry, F.H., D.W. Lee, and A.R. Bertolino. 1977. Progress in Atlantic bluefin tuna aging attempts. International Commission for the Conservation of Atlantic Tunas. Collective Volume of Scientific Papers 6(67):305-317.

Chadwick, E.M.P. 1976. A comparison of growth and abundance for tidal pool fishes in California and British Columbia. J. Fish Biol. 8(1): 27-34.

Galtsoff, P.S. 1952. Staining of growth rings in the vertebrae of tuna *(Thunnus thynnus).* Copeia 1952(2):103-105.

Menon, M.D. 1950. The use of bones, other than otoliths, in determining the age and growth-rate of fishes. J. du Cons. Int. Explor. Mer 16(3):311-335.

Mather, F.J., III, and H.A. Schuck. 1960. Growth of bluefin tuna of the western north Atlantic. Fish. Bull. Fish and Wildl. Ser. 61(179):39-52.

Allyn G. Johnson, *SEFC, National Marine Fisheries Service, NOAA Panama City, Laboratory, 3500 Delwood Beach Road, Panama City, FL 32407.*